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Immunity *25*, December 2006 ©2006 Elsevier Inc. DOI 10.1016/j.immuni.2006.11.002

Rulers over Randomness: Stroma Cells Guide Lymphocyte Migration in Lymph Nodes

How is the amoeboid movement of lymphocytes in secondary lymphoid organs orchestrated? In this issue of *Immunity*, Bajénoff et al. (2006) demonstrate that stromal cell networks serve as guidance structures that direct and limit the migration of B and T cells in lymph nodes.

Secondary lymphoid organs (SLOs), including lymph nodes (LN), are hubs of leukocyte trafficking and provide structural platforms for information transfer between innate and adaptive immune cells (von Andrian and Mempel, 2003). SLOs have evolved to optimize the likelihood that rare lymphocytes with unique antigenic specificity encounter their cognate antigen in the appropriate immunogenic or tolerogenic context. Critical to the success of this system are search strategies that maximize the exposure of each individual lymphocyte to the largest possible number of antigen-presenting cells (APCs). To this end, naive lymphocytes must incessantly recirculate between blood and SLOs, spending typically less than a day in each organ and migrating actively within the interstitial space to query vast numbers of APCs along their path.

Recent observations with confocal and multiphoton intravital microscopy of fluorescently tagged immune cells in their natural habitat have reshaped our understanding of how adaptive immune responses are initiated and regulated in LNs. Initial studies had noted that T and B cell migration was not only more dynamic than anticipated but also apparently random in direction. The observed migratory patterns seemed best described by a refined version of the random-walk model, which is classically used to describe the diffusion of particles (Miller et al., 2002). There are, however, several caveats that must be kept in mind when fluorescence-based intravital imaging studies are performed in SLOs: the relatively small sample volume that can be imaged within the organ; the limited duration of continuous recordings (typically one hour or less); the small fraction of any particular cell population that can be visualized; and the inability to simultaneously detect and discriminate between the many diverse constituents of the LN microenvironment (e.g., nonfluorescent immune cells, blood vessels, stromal cells, extracellular matrix, etc.).

Despite these uncertainties, motility data have been interpreted as if lymphocytes were migrating in a homogenous matrix (composed of invisible lymphocytes) without physical obstacles or a stationary adhesion substrate. The migrating cells' behavior was likened to that of autonomous agents that follow an intrinsic search algorithm (Miller et al., 2003). Characteristic aspects of this behavior were minute-long phases of migration along a fairly straight path interrupted by short migratory pauses during which cells were believed to reorient their locomotion machinery. Calculations have shown that the high motility of randomly migrating T cells, combined with the ability of dendritic cells (DCs) to maximize the volume within which they display antigens by sweeping their expansive dendrites in the surrounding space, allows for a high scanning efficiency. Published estimates range from 500 to as many as 5000 T cells that may contact individual DCs per hour (Breart and Bousso, 2006).

Although the erratic movement of interstitial lymphocytes negates a simple role for diffusible chemoattractants exerting long-distance directional control over lymphocyte migration, several observations demand the existence of hidden rules that restrict migratory randomness. A striking case in point is the strict separation of B follicles and the T cell area. B and T cells migrate vigorously in their respective domains, but rarely trespass into each other's territory. Experiments in knockout mice have established long ago that chemokines are essential for this compartmentalization, and recent imaging studies in LNs show that chemokines control T and B cell migration also at the single-cell level, at least under certain conditions. For example, when intrafollicular B cells encounter antigen, they upregulate the chemokine receptor CCR7, thus sensitizing themselves to the abundant CCR7 ligands in the T cell area, which attract them to the follicle border (Okada et al., 2005). Similarly, activation of $CD4^+$ T cells induces CXCR5, which allows T cells to access the B cell follicle to provide B cell help. Moreover, naive $CD8^+$ T cells rapidly upregulate CCR5 when they enter LNs that drain a vaccination site. Antigen-presenting DCs are prompted by activated $CD4^+$ T cells to secrete CCR5 ligands. This increases the contact frequency between $CCR5^+$ $CD8^+$ T cells and the "helped" DCs (Castellino et al., 2006).

In vitro, chemokines diffusing in solution or across gels or membranes establish concentration gradients that

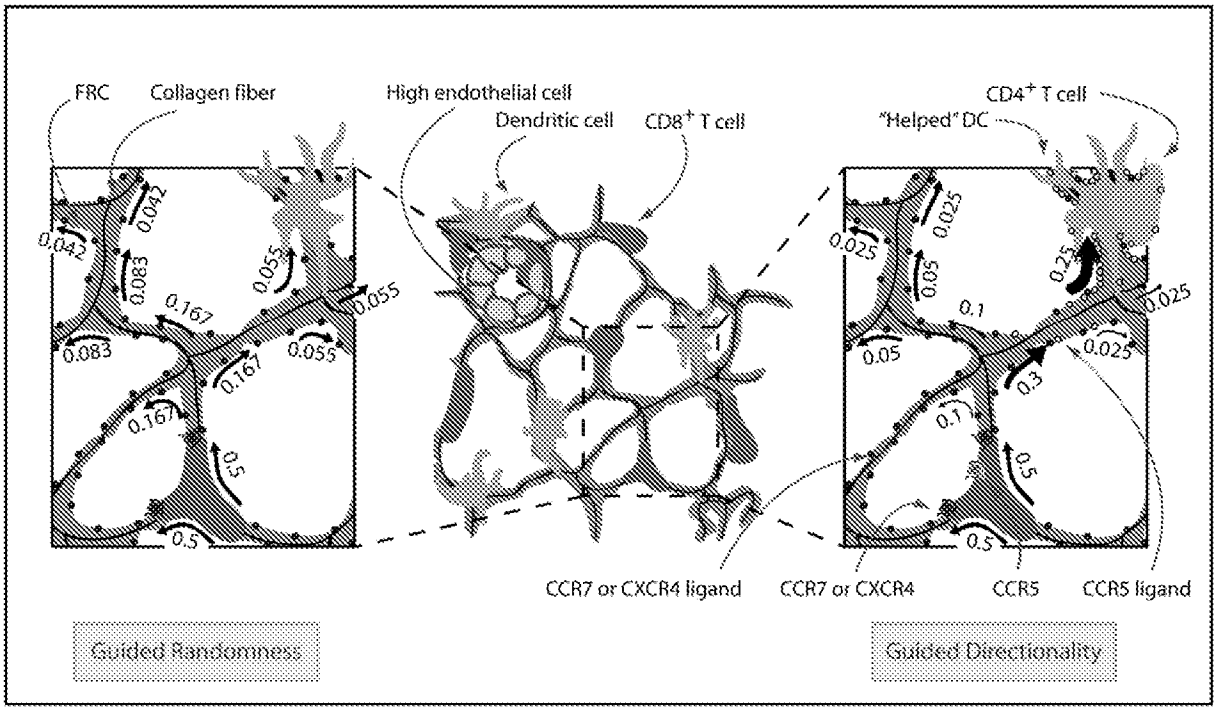

Figure 1. A Guidance Model for Interstitial T Cell Migration in Lymph Nodes

(Center) Upon exit from high endothelial venules (upper left) and the surrounding perivenular space, lymphocytes stay in continuous contact with fibroblastic reticular cells (FRCs). These stromal cells ensheath the collagen-fiber scaffold and provide an adhesion substrate as well as a guidance structure, thus enabling and restricting T cell migration.

(Left) In the steady-state setting, homeostatic chemoattractants (possibly chemokine agonists for CCR7 and/or CXCR4) produced by and deposited on the surface of FRCs provide haptokinetic stimuli for T cell migration. In the absence of inflammatory challenges, T cells are approximately equally likely to turn in either direction at every furcation of the network. This results in "guided randomness" of migration, at least within the boundaries of the stromal network. Numbers and arrows denote the probability of the T cell (red) in the starting position to pass along a particular branch of the "downstream" network.

(Right) During immune responses, $CD8^+$ T cells acquire sensitivity for additional chemoattractant cues, such as CCR5 ligands, which are produced when helper T cells interact with DCs presenting cognate antigen. The localized pattern of deposition of these chemokines in the vicinity of "helped" DCs generates a local directional bias at FRC furcations, thus enhancing the probability (indicated by thicker arrows) that $CCR5^+$ T cells interact with foreign antigen-presenting DCs.

attract lymphocytes. However, given the microanatomic and biochemical complexity of lymphoid tissues, it seems at best questionable that freely diffusible chemokine gradients could form and remain sufficiently undisturbed to exert robust control over cell migration in vivo. Chemokines can attach to cell surfaces and extracellular-matrix molecules by binding certain glycosaminoglycans (Rot and von Andrian, 2004). Thus, chemokines may be immobilized on hard-wired guidance structures to elicit haptotactic or haptokinetic lymphocyte migration in SLOs. However, the nature and existence of such guidance structures has remained conjecture so far.

Using high-resolution electron and light microscopy, Bajénoff et al. (2006) report in the current issue of *Immunity* direct evidence for an intricate lymphocyte guidance system in LNs. Intravital microscopy showed that both T and B cells migrate almost exclusively along networks of two stromal cell types, fibroblastic reticular cells (FRCs) and follicular dendritic cells (FDCs), which reside in the T and B cell areas, respectively. This discovery does not contradict earlier imaging studies in LNs, but it changes their interpretation and mandates a revisitation of older concepts (Gretz et al., 1997).

A body of earlier work mainly by the groups of Anderson and Shaw describes the ultrastructural details of the "reticular network" permeating the T cell area and, at lower density, the B follicles of LNs (Gretz et al., 1997). Each branch of the FRC network has a core of collagen fibers surrounded by extracelluar-matrix molecules, a basement membrane, and a sleeve of stromal cells, the FRCs. The space between collagen fibers and the basement membrane is accessible to small molecules, which can be channeled from the subcapsular sinus to perivascular spaces around high endothelial venules (HEVs) (Gretz et al., 1997). DCs in the T cell area occupy small gaps between FRCs and can thus acquire lymph-borne molecules. Anderson and Shaw speculated that T cells may use FRCs as adhesive scaffolds for migration. This would conveniently guide them toward DCs that present lymph-borne antigens.

The work by Bajénoff et al. (2006) now puts this idea on firm footing. The authors performed multiphoton intravital microscopy in popliteal LNs of anesthetized mice that expressed enhanced green fluorescent protein (EGFP) in nonhematopoietic cells, including endothelial cells (ECs), FRCs, and FDCs. Interactions of $EGFP^+$ stromal elements with adoptively transferred T and B cells labeled in a complementary fluorescent color were then dynamically visualized. Upon exit from HEVs, lymphocytes did not immediately enter the free interstitial space

of the LN cortex, but were first retained in perivenular channels (PVCs) between ECs and a surrounding layer of FRCs, as suggested by earlier static-imaging experiments (Gretz et al., 1997). T cells preferentially exited the PVCs at certain hot spots, termed "exit ramps," and immediately attached to FRC fibers along which they began to migrate. When the migrating cells reached fiber bifurcations, they would briefly hesitate and then continue along one of the branches. Importantly, changes in T cell direction rarely occurred in the absence of fiber branches, suggesting that the previously observed stopping and turning of T cells is imposed by the FRC scaffold.

Analogous observations were also made with B cells migrating along FDC networks in the B follicles. Interestingly, the FRC reticulum, as defined by expression of the ERTR-7 antigen, is prominent in the T cell zone but sparse in B follicles. The findings by Bajénoff et al. (2006) suggest that T cells depend on FRC fibers to migrate, which could explain the territorial exclusion of T cells from follicles.

Given these new insights, we propose a hypothetical picture of T cell migration in LNs (Figure 1) governed by the following simple rules: (1) T cell migration depends on and is restricted to the presence of the FRC reticulum; (2) the (as yet unidentified) molecular cues provoking the incessant motility of T cells are probably associated with the outer surface of FRCs, possibly in form of immobilized chemokines; (3) the directionality of baseline T cell migration along FRCs is essentially random; (4) activated DCs may modulate nearby segments of the FRC network, thereby generating hot spots of enhanced attractiveness and introducing a directional bias that restricts T cell motility (Castellino et al., 2006; Mempel et al., 2004); and (5) changes in chemoattractant receptor expression allow T cells to newly sense directional signals that override or compete with the constitutive promoter(s) of random walk. One can envision the set of possible paths an individual T cell can take along the FRC network from a particular starting position as a decision tree. In the steady state, the probabilities to arrive at the different downstream locations at each furcation are evenly distributed, creating a probability map with evenly decreasing values away form the starting point (Figure 1). The probability map is, however, asymmetric at the borders to other LN microenvironments and in the vicinity of competing local sources of chemoattraction because the likelihood of migration along certain branches is favored over the others at individual furcations (Figure 1).

Clearly, the general applicability of this model will require further experimental testing. Many questions remain to be answered: Do lymphocytes compete with each other and/or other cell types for access to reticular networks, and, if so, what are the rules of this competition? What is the role of adhesion and signaling molecules in this setting, and how is lymphocyte behavior altered under circumstances leading to impaired adhesion? How does a T cell integrate the multitude of external cues it encounters? Finally, LNs also recruit DCs and other inflammatory cells that express distinct sets of traffic molecules. Whether and how these cells are guided by reticular fibers and how all of these events are modulated in pathological settings remains to be determined.

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